Leptin and leptin receptor expression in skeletal muscle and adipose tissue in response to in vivo porcine somatotropin treatment[1,2]

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ABSTRACT: The present study was performed to examine the response of leptin and leptin receptor (Rb) genes to porcine somatotropin (pST) stimuli in finishing pigs. Twelve crossbred barrows (Yorkshire × Landrace) were used in this study. Animals were individually fed a basal diet containing 18% CP, 1.2% lysine, and 3.5 Mcal of DE/kg ad libitum (as-fed basis). At 90 kg, six pigs were treated with daily injections of recombinant pST (10 mg) in sterile bicarbonate buffer, whereas the other six pigs were injected with sterile bicarbonate buffer (controls). With initiation of pST treatment, the quantity of feed offered was 85% of calculated ad libitum intake based on BW and adjusted every 3 d. Diet restriction was designed to correct for the effects of the known inhibition in feed intake because of pST treatment in swine. Animals were maintained on treatment for 2 wk. A blood sample was obtained from each pig on d 14 of treatment, 6 h after pST injection. Tissue samples were collected on d 15, frozen in liquid N_2, and stored at –80°C before analysis for mRNA abundance. Total RNA was amplified by reverse transcription (RT) PCR with subsequent quantification of transcripts by capillary electrophoresis with laser-induced fluorescence detection. Samples included outer subcutaneous adipose tissue (OSQ), middle subcutaneous adipose tissue (MSQ), leaf fat (LF), liver, latissimus dorsi (LD), and biceps femoris (BF). Restricted feeding resulted in no change in BW of control pigs, whereas pST treatment increased BW by 6.9 ± 0.5 kg ($P < 0.001$). Treatment with pST produced a 12-fold increase in serum ST concentration relative to control pigs ($P < 0.002$). Serum leptin concentration was increased by 17% in swine treated with pST relative to control pigs ($P < 0.011$). Leptin mRNA abundance was increased in liver by pST treatment ($P < 0.05$). Administration of pST decreased leptin Rb (Ob-Rb) mRNA abundance by 27% in liver ($P < 0.044$) and by 49.5% in OSQ ($P < 0.025$) relative to controls. The present data suggest that pST does not affect leptin expression independent of dietary intake because the restricted feeding regimen used in the present study precluded detection of major change in leptin gene expression. Changes in Ob-Rb mRNA abundance by pST treatment indicate that ST or the metabolic adaptations to ST have a role in regulating Ob-Rb expression.

Key Words: Leptin, Leptin Receptor, Somatotropin, Swine

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Introduction

Porcine somatotropin (**pST**) has been demonstrated in swine to have dramatic effects on adipose tissue accretion, metabolism, and gene expression (Etherton, 2004). The processes affected by pST are many of the same that are affected by the hormone leptin. Leptin is a hormone that is produced and secreted by adipose tissue with numerous effects on metabolism, immune response, reproduction, and feeding behavior (Barb et al., 2001).

The regulation of leptin expression has been well characterized in vitro; however, characterization of the in vivo regulation has been limited in the pig. Changes in leptin mRNA abundance have been correlated with adiposity (Leininger et al., 2000), whereas differences in tissue leptin mRNA abundance have been associated with differences in serum leptin concentrations between lean and genetically obese pigs (Ramsay et al., 1998). Very few studies have examined the role of specific hormones in the in vivo regulation of leptin mRNA abundance in the pig. In numerous species, ST has

[1]Mention of a trade name, vendor, proprietary product, or specific equipment is not a guarantee or a warranty by the USDA and does not imply an approval to the exclusion of other products or vendors that also may be suitable.

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Table 1. Composition of diet[a]

Ingredient	%, as-fed basis
Corn	61.8
Soybean meal, 48% CP	18.0
Dried skim milk	12.0
Calcium carbonate	2.5
Sodium phosphate, monobasic	2.5
Corn oil	2.0
Iodized salt	0.5
Lysine hydrochloride	0.25
Mineral premix, swine complete mix[b]	0.2
Vitamin mix[b]	0.2
Selenium premix[c]	0.05

[a]Calculated nutrient composition: 18% CP, 1.2% lysine, and 3.5 Mcal of DE/kg (as-fed basis).
[b]For composition of mineral and vitamin premixes, see Campbell et al. (1988).
[c]Provided 22 μg of Se/kg of diet.

been demonstrated to alter leptin mRNA abundance in subcutaneous adipose tissue (Isozaki et al., 1999; Houseknecht et al., 2000) including swine (Spurlock et al., 1998). Nonetheless, the relationship of tissue leptin mRNA abundance to serum leptin has not been evaluated in swine in response to pST administration.

Leptin functions through a receptor (**Rb**) with multiple splice sites. The long form of the Rb is the only one to demonstrate signaling capability consistently. Leptin Rb (**Ob-Rb**) mRNA (long form) has been detected in a variety of porcine tissues, including adipose tissue (Lin et al., 2000). Regulation of the Ob-Rb has not been well characterized for any species. The present study was designed to examine the in vivo response of leptin and Ob-Rb genes to pST administration.

Materials and Methods

Twelve crossbred barrows (Yorkshire × Landrace) were individually penned in environmentally controlled housing at 70 kg. Animals were individually fed a basal diet ad libitum containing 18% CP, 1.2% lysine, and 3.5 Mcal of DE/kg (Table 1).

At 90 kg, six randomly selected pigs were treated with daily injections of sterile recombinant pST (10 mg; Southern Cross Biotech, Toorak, Victoria, Australia) in sodium bicarbonate buffer (pH 9.4). This dose was based on previous studies in pigs of this size (Campbell et al., 1988; Caperna et al., 1990; Steele et al., 1995). The other six pigs served as controls and were injected with sterile bicarbonate buffer alone. Injections (1.0 mL) were performed into the extensor neck muscles daily between 0800 and 0830. Fresh feed was presented at 0900.

With initiation of pST treatment, the feed was offered at 85% of calculated ad libitum intake (ARC, 1981) based on BW and adjusted every 3 d. Animals were maintained on treatment for 2 wk. A blood sample was obtained from each pig on d 14 of treatment at 1500. Animals were euthanized on d 15 at 0800.

Somatotropin has been demonstrated to dramatically shift metabolic variables to promote muscle accretion and decrease adipose accumulation. In association with this shift, animals experience a decrease in feed intake but an increase in feed efficiency (Etherton, 2004). In the present study, feed restriction was used to specifically exclude any differences in feed intake caused by pST treatment as contributing to changes in leptin or Ob-Rb mRNA abundance. Feed withdrawal has been demonstrated to decrease leptin mRNA abundance in swine (Spurlock et al., 1998; Salfen et al., 2003), whereas feed restriction to 80% of ME intake decreased serum leptin concentrations by approximately 37% in gilts (Louveau et al., 2000). Thus, exclusion of feed intake as a component of the pST response would permit examination of the data in terms of a specific response to the pST treatment.

Various tissues were acquired following euthanasia by electrical stunning and exsanguination according to procedures approved by the Institutional Area Animal Use and Care Committee. Dorsal subcutaneous adipose tissue samples were collected from between the second and fourth thoracic vertebrae, and subsequently, outer (**OSQ**) and middle (**MSQ**) layers were separated, diced, and frozen in liquid N_2. In addition, samples of liver, leaf (perirenal) fat (**LF**), latissimus dorsi (**LD**), and biceps femoris (**BF**) muscle were collected, diced, and frozen in liquid N_2. Outer and MSQ were separated for analysis because of their known differences in metabolic activity, whereas LF has a different metabolic profile from subcutaneous adipose tissue (Anderson et al., 1972; Rule et al., 1989; Budd et al., 1994). The LD and BF were selected, as they represent large muscles in economically important regions that differ in their response to pST (Ono et al., 1995).

Hormone Analyses

Concentrations of pST were determined by a homologous RIA (Linco Research, Inc., Chesterfield, MO). Intra-assay CV was 6.03% for pST. Serum leptin was assayed using a heterologous RIA kit (Linco Research, Inc.). Human leptin standards were replaced with recombinant porcine leptin standards. Recombinant porcine leptin was obtained from A. Gertler (Rehovot, Israel; Raver et al., 2000). Cross-reactivity for the recombinant porcine leptin in the multi-species leptin RIA was 58%. Intra-assay CV was 4.94%, and the inter-assay CV was 10.3%. The minimal detectable concentration was 0.1 ng/tube, recovery of added leptin was >93% across the range of 1 to 20 ng of leptin added.

Gene Expression Analyses by Reverse Transcription PCR

Total RNA was isolated using TRI reagent according to the manufacturer's protocol (Sigma-Aldrich, St.

Louis, MO). Integrity of RNA was assessed via agarose gel electrophoresis, and RNA concentration and purity were determined spectrophotometrically using 260 and 280 nm absorbance measurements. Reverse transcription (**RT**) reactions (20 μL) consisted of 1 μg of total RNA, 50 U of SuperScript II reverse transcriptase (Invitrogen/ Life Technologies, Carlsbad, CA), 40 U of an RNAse inhibitor (Invitrogen/Life Technologies), 0.5 mmol/L of dNTP, and 100 ng of random hexamer primers. Polymerase chain reaction was performed in 25 μL containing 20 mmol/L of Tris-HCl (pH 8.4), 50 mmol/L of KCl, 1.0 μL of the RT reaction, 1.0 U of Platinum Taq DNA polymerase (Hot Start; Invitrogen/Life Technologies), 0.2 mmol/L of dNTP, 2.0 mmol/L of Mg++ (Invitrogen/Life Technologies), 10 pmol each of the leptin and Ob-Rb-specific primers, and 10 pmol of an appropriate mixture of primers and competimers specific for 18S rRNA (QuantumRNA Universal 18S Internal Standard; Ambion, Inc., Austin, TX). Thermal cycling protocol was as follows: 1 cycle at 94°C for 2 min, followed by 30 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 8 min.

The following primers were used for generating 348 base-pair PCR products corresponding to a portion of the pig leptin coding sequence: 5′- TGACACCAAAAC CCTCATCA -3′ (forward), 5′- GCCACCACCTCTGTG GAGTA -3′ (reverse). The primers for the long form Ob-Rb were used to generate a 396-basepair product: 5′- C AGTGACATTTGGCCCTCTT -3′ (forward), 5′- AGGC CTGGGTTTCTATCTCC -3′ (reverse). The leptin and Ob-Rb amplicons were excised from an agarose gel, reamplified, and run through a GenElute PCR clean-up kit (Sigma-Aldrich). The amplicons were subsequently sequenced to confirm identity using automated fluorescent DNA sequencing (ABI 310; Perkin-Elmer Applied Biosystems, Foster City, CA).

Capillary Electrophoresis with Laser-Induced Fluorescence Detection

Aliquots (2 μL) of RT-PCR samples were diluted 1:100 with deionized water before capillary electrophoresis with laser-induced fluorescence detection (**CE/LIF**). A detailed description and validation of the CE/LIF technique used in this study for quantitative analysis of gene expression was reported previously (Richards and Poch, 2002). Briefly, a P/ACE MDQ series capillary electrophoresis instrument (Beckman Coulter, Fullerton, CA) equipped with an argon ion LIF detector was used. Capillaries were 75 μm i.d. × 32 cm μSIL-DNA (Agilent Technologies, Folsom, CA). EnhanCE dye (Beckman Coulter) was added to the DNA separation buffer (Sigma-Aldrich) to a final concentration of 0.5 μg/mL. Samples were loaded by electrokinetic injection at 3.5 kV for 3.5 s and run in reverse polarity at 8.1 kV for 5 min. Integrated peak area for the PCR products separated by CE was calculated using P/ACE MDQ software (Beckman Coulter).

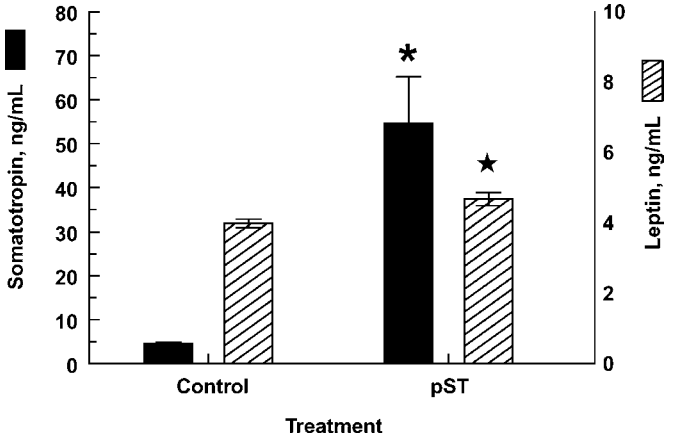

Figure 1. Serum porcine ST (pST; solid bars) and leptin (hatched bars) concentrations in feed-restricted swine following 14 d of pST administration (10 mg/d). Data (ng/mL) are expressed as means ± SEM. The asterisk indicates that the mean pST concentration differs from that of control (buffer-injected) swine, $P < 0.002$ (n = 6). The star indicates that the mean leptin concentration differs from that of the control (buffer-injected) swine, $P < 0.011$ (n = 6).

Quantification of Messenger Ribonucleic Acid Abundance

Relative mRNA abundance was determined as the ratio of integrated peak area for each individual gene PCR product relative to that of a coamplified 18S internal standard (QuantumRNA Universal 18S Internal Standard; Ambion, Inc). Values are presented as the mean ± SEM of four to six individual determinations.

Statistical Analyses

Data were analyzed by one-way ANOVA using SigmaStat software (SPSS Science, Chicago, IL). Mean separation was done using Student-Newman-Keuls test, and means were defined as significantly different at $P < 0.05$.

Results

Cumulative feed intake did not differ ($P = 0.240$) between the two groups (38.58 ± 0.22 kg vs. 38.74 ± 0.15 kg for control vs. pST, respectively). Restricted feeding resulted in no change ($P = 0.394$) in BW of control pigs, but treatment with recombinant pST for 14 d resulted in an average increase ($P < 0.001$) in BW of 6.9 ± 0.5 kg relative to control pigs. Treatment with pST resulted in a 12-fold increase ($P < 0.002$) in serum ST relative to control pigs (Figure 1). Serum leptin concentration was increased ($P < 0.011$) by 17% in swine treated with pST relative to control pigs (Figure 1).

Leptin mRNA abundance was increased ($P < 0.05$) in liver by pST treatment (Figure 2). No other tissue

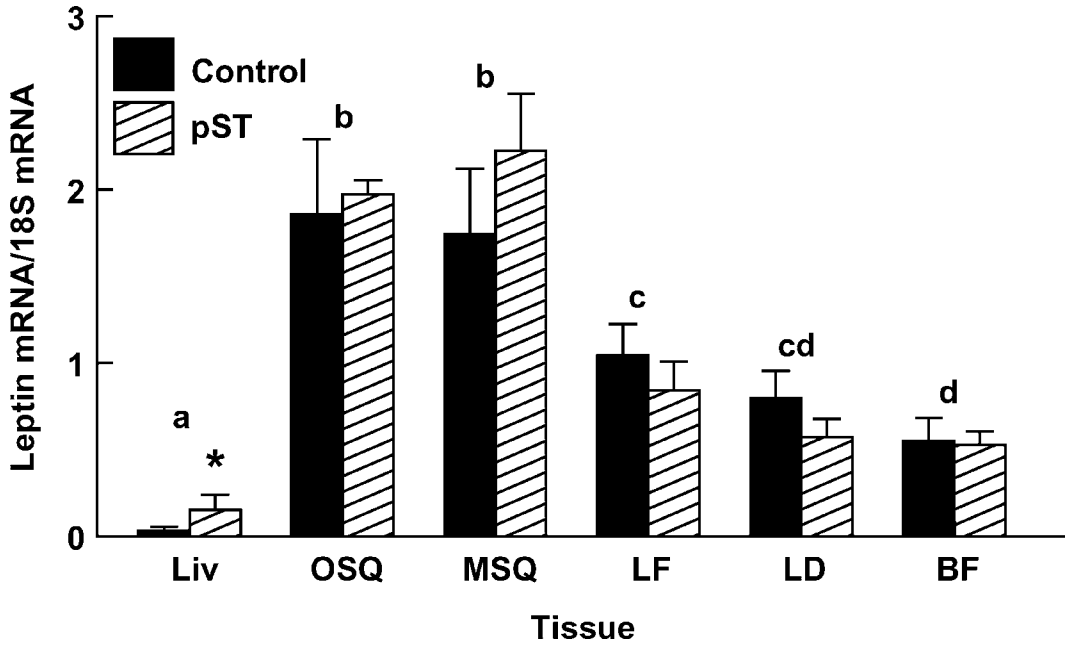

Figure 2. Relative leptin mRNA abundance in tissues from feed-restricted swine following 14 d of porcine ST (pST) administration (10 mg/d) followed by extraction for total RNA and subsequent reverse transcription PCR analysis for leptin mRNA abundance. Data are expressed as the mean ratios ± SEM of specific leptin mRNA:18S mRNA for six pigs in each group. The asterisk indicates that the mean differs from that of corresponding control (buffer-injected) swine, $P < 0.05$ (n = 6). Means that do not have a common letter differ, $P < 0.05$ (n = 6). Liv = liver, OSQ = outer subcutaneous adipose tissue, MSQ = middle subcutaneous adipose tissue, LF = leaf fat, LD = latissimus dorsi, and BF = biceps femoris.

examined was affected by pST treatment ($P = 0.428$ for LF, $P = 0.360$ for MSQ, $P = 0.699$ for OSQ, $P = 0.248$ for LD, and $P = 0.890$ for BF). Pooling of data within tissue for comparison of leptin mRNA abundance among tissues demonstrated that mRNA abundance was greatest in subcutaneous adipose tissues and least in liver ($P < 0.001$). Leptin mRNA abundance in LF also was greater ($P < 0.001$) than in liver or BF but not different ($P = 0.097$) from LD.

Leptin Rb mRNA abundance was decreased by pST administration in liver and OSQ (Figure 3). Leptin Rb mRNA abundance was decreased ($P < 0.044$) by 27% in liver following pST treatment, whereas it was decreased ($P < 0.025$) by 49.5% in OSQ with pST administration. However, transcription of the Ob-Rb gene was not affected by systemic pST treatment in LD ($P = 0.853$), BF ($P = 0.124$), LF ($P = 0.610$), or MSQ ($P = 0.485$). Pooling of data within tissue for comparison of Ob-Rb mRNA abundance among tissues showed that Ob-Rb mRNA concentrations were greatest in skeletal muscles and liver and least in all of the adipose tissues ($P < 0.001$).

Discussion

Swine treated with pST gained BW during the 14 d of treatment, whereas controls gained no BW because of the diet restriction to 85% of predicted ad libitum intake. This BW gain confirmed that pST had systemic anabolic effects in the present study. Feed restriction has previously been demonstrated to not alter serum leptin concentrations in cycling gilts (Almeida et al., 2001). The increase in serum leptin in pST-treated swine compared with control animals consuming equivalent amounts of feed implies that pST has an effect on leptin secretion independent of the effect of pST on feeding behavior. The present study did not take into account the potential pulsatility in swine serum leptin as effectively demonstrated by Whisnant and Harrell (2002). Therefore, it is unknown whether the differences between controls and pST-treated swine are the minimum or maximum differential response. Whether this slight shift in serum leptin concentration may be of biological significance is unknown.

Previous studies in humans and rodents have demonstrated that somatotropin concentrations can be correlated with increased (Iglesias et al., 2002), decreased (Eden-Engstrom et al., 2003; Malmlof and Johansen, 2003), or normal (Boni-Schnetzler et al., 1999; Bolanowski et al., 2002; Schulz et al., 2002) serum leptin concentrations. The serum leptin response to pST may depend on the status of carcass adipose mass, serum insulin, serum glucocorticoid, renal state, pre-experimental ST concentration, timing of collection, or other factors.

The observed shift in serum leptin with pST treatment could not be related to any change in adipose

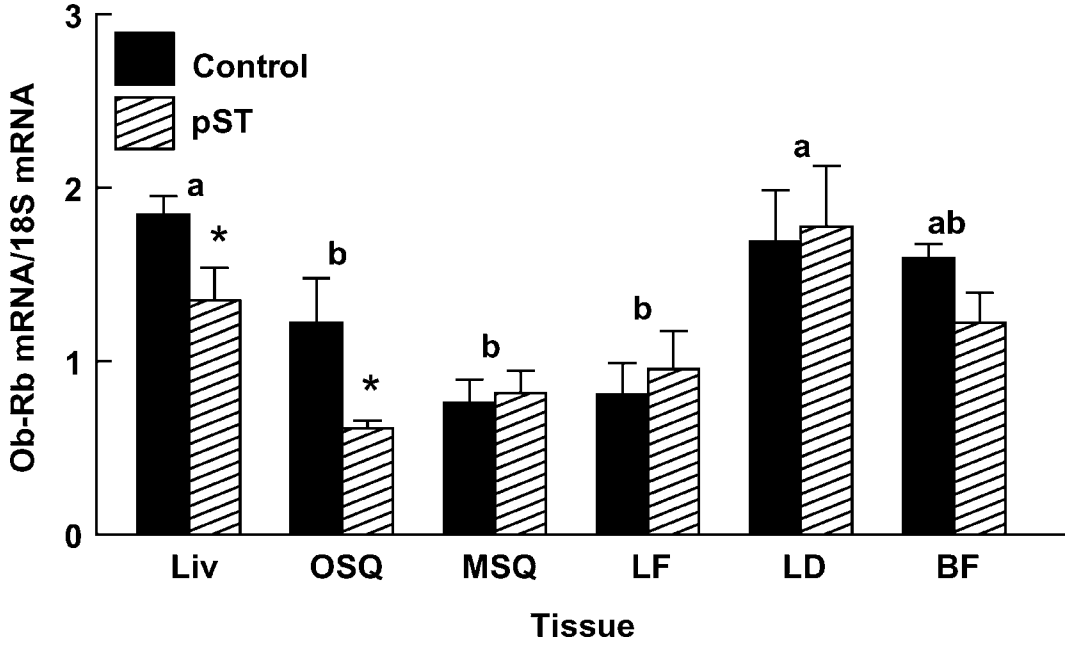

Figure 3. Relative long-form leptin receptor (Ob-Rb) mRNA abundance in tissues from feed-restricted swine following 14 d of porcine ST (pST) administration (10 mg/d) followed by extraction for total RNA and subsequent reverse transcription PCR analysis for leptin mRNA abundance. Data are expressed as the mean ratios ± SEM of specific Ob-Rb mRNA:18S mRNA for six pigs in each group. The asterisks indicate that the means are different than that of control (buffer-injected) swine, $P < 0.05$ (n = 6). Means that do not have a common letter differ, $P < 0.05$ (n = 6). Liv = liver, OSQ = outer subcutaneous adipose tissue, MSQ = middle subcutaneous adipose tissue, LF = leaf fat, LD = latissimus dorsi, and BF = biceps femoris.

tissue leptin mRNA abundance. Previous research in swine has demonstrated that serum leptin is elevated when adipose tissue mRNA abundance is elevated within obese pigs relative to lean pigs (Ramsay et al., 1998). Nonetheless, results of the present study suggest that leptin mRNA abundance in adipose tissue does not necessarily correlate with changes in serum leptin following pST treatment.

Spurlock et al. (1998) reported that pST administration can decrease leptin mRNA abundance by 50% in adipose tissue of feed-restricted pigs, suggesting that pST-treated pigs have less stimulus for satiety and are thus hungrier; this would indicate that pST-treated swine would consume greater quantities of feed when re-fed. In contrast, pST-treated swine consume less feed than control swine in most studies (Etherton, 2004). Equalizing the intake of control animals with pST-treated swine in the present experiment eliminated the suppression in leptin mRNA abundance. The additive suppressive effect of pST with the feed withdrawal response on leptin, as reported by Spurlock et al. (1998) might be due to the high concentration of free fatty acids in that study. Free fatty acids have been demonstrated to down-regulate leptin expression (Rentsch and Chiesi, 1996; Shintani et al., 2000; Cammisotto et al., 2003). Free fatty acid concentrations in pST-treated pigs in the present study were 17% (data not presented) of the concentration in the feed-restricted, pST-treated pigs in the Spurlock et al. (1998) study. The extremely high concentration of free fatty acids in the feed-restricted, pST-treated swine may account for the pST inhibition of leptin mRNA abundance in the study of Spurlock et al. (1998) vs. the absence of a difference in the restricted-fed pigs in the present study.

Leptin mRNA abundance was greatest in subcutaneous adipose tissue, with no differences between middle and outer layers in our feed-restricted swine. Leptin mRNA abundance has been correlated with adipocyte size (Zhang et al., 2002; Guo et al., 2004), but OSQ and MSQ have been reported to have similar cellularity (Anderson et al., 1972). Thus, similar abundance of leptin mRNA would be predicted for OSQ and MSQ. The leptin mRNA abundance in LF was approximately 50% of that in subcutaneous adipose tissue. Previous work with human adipose tissue has demonstrated that the adipocytes from internal sites of adipose accretion have lower leptin mRNA abundance than subcutaneous adipose tissue (Montague et al., 1997).

Under our experimental conditions, leptin mRNA was detectable in skeletal muscle with abundance approaching that in LF. Leptin mRNA has previously been detected in skeletal muscle (Wang et al., 1998). The primary source of this leptin mRNA is most likely the intramuscular adipose tissue, which was not dissected from the muscle in the present study.

Liver leptin mRNA abundance was least in the tissues examined. Leptin mRNA has been previously detected in mammalian hepatic tissue (Otte et al., 2004), but it was primarily restricted to the stellate cell population (Potter et al., 1998). Somatotropin has been previously demonstrated to up-regulate leptin mRNA abundance in chicken liver (Ashwell et al., 1999); however, leptin mRNA abundance in the pig liver is extremely low and the biological significance of this up-regulation is unknown.

Little is understood of the regulation of the Ob-Rb in peripheral tissues for any species. The majority of research on the porcine Ob-Rb has focused simply on detection of the Rb (Czaja et al., 2002) or on its regulation at the pituitary gland and hypothalamus (Lin et al., 2003). Previous studies using adipose tissue explants have demonstrated an inhibitory effect of pST on total Ob-Rb mRNA abundance in MSQ explants in vitro (Ramsay and Richards, 2004). This inhibitory effect could not be replicated in MSQ by in vivo administration of pST. This result may be a consequence of interaction with the hormonal milieu in vivo relative to the controlled environment of tissue culture or it may be a consequence of differences in the regulation of the long form of the Ob-Rb relative to the total population of Ob-Rb. Differential regulation of Ob-Rb isoforms has been previously demonstrated in rat hypothalamus (Denis et al., 2003) and testis (Tena-Sempere et al., 2001).

In vivo treatment with pST resulted in lower abundance of long-form Ob-Rb mRNA in liver and OSQ relative to tissues from control animals consuming similar quantities of feed. Any shift in Ob-Rb expression in the liver could be of significance, as the liver has a significant role in regulating energy metabolism. Although leptin has not yet been demonstrated to alter hepatic metabolism in the pig (Fernandez-Figares et al., 2004; Raman et al., 2004), leptin has been shown to inhibit dexamethasone-induced hepatic IGF-1 mRNA abundance in vitro (Ajuwon et al., 2003).

Leptin Rb mRNA abundance was decreased by pST to a greater extent in OSQ than in the liver. Changes in the relative expression of the Ob-Rb by in vivo pST treatment would imply altered sensitivity to leptin and perhaps a decreased capacity to inhibit lipogenesis and promote lipolytic activity of the porcine adipocyte (Ramsay, 2000, 2003a). These same actions of leptin may be superseded by similar actions of pST on the pig adipocyte (Etherton, 2004). We hypothesize that a decrease in leptin binding and internalization may contribute to the elevation in the serum leptin concentration following pST treatment.

Abundance of the Ob-Rb mRNA was as great in skeletal muscles as liver, with less abundance in adipose tissues. Previous studies have detected the presence of Ob-Rb mRNA in muscle cells (Berti and Gammeltoft, 1999; Fruhbeck et al., 1999) and the ability of leptin to alter glucose, fatty acid, and amino acid metabolism to promote muscle anabolism (Ceddia et al., 1998; Berti and Gammeltoft, 1999; Ramsay, 2003b). Expression of the Ob-Rb in skeletal muscle and the known metabolic effects of leptin on muscle from studies in other species imply that leptin may function in pig skeletal muscle to alter metabolic activity.

Leptin is a hormone produced by pig adipose tissue that can affect feeding behavior, animal health, and reproduction. Studies in pigs have demonstrated that leptin can decrease feed intake. This study attempted to determine whether expression of leptin and its Rb can be manipulated by hormonal mechanisms in finishing swine by using pST treatment. The data indicate that circulating leptin concentrations are stimulated by pST when feed intake is equivalent. Second, expression of the Ob-Rb gene also is responsive to hormonal stimuli in finishing swine. In contrast, the leptin gene does not seem responsive to somatotropin treatment when the effects of pST to decrease feed intake are taken in account. These results suggest the potential to manipulate the efficiency of feeding behavior in finishing animals by altering expression of genes associated with feed intake and metabolism.

Literature Cited

Ajuwon, K. M., J. L. Kuske, D. Ragland, O. Adeola, D. L. Hancock, D. B. Anderson, and M. E. Spurlock. 2003. The regulation of IGF-1 by leptin in the pig is tissue specific and independent of changes in growth hormone. J. Nutr. Biochem. 14:522–530.

Almeida, F. R., J. Mao, S. Novak, J. R. Cosgrove, and G. R. Foxcroft. 2001. Effects of different patterns of feed restriction and insulin treatment during the luteal phase on reproductive, metabolic, and endocrine parameters in cyclic gilts. J. Anim. Sci. 79:200–212.

Anderson, D. B., R. G. Kauffman, and L. L. Kastenschmidt. 1972. Lipogenic enzyme activities and cellularity of porcine adipose tissue from various anatomical locations. J. Lipid Res. 13:593–599.

ARC. 1981. The Nutrient Requirements of Pigs. Commonwealth Agricultural Bureaux, Slough, UK.

Ashwell, C. M., J. P. McMurtry, X. H. Wang, Y. Zhou, and R. Vasilatos-Younken. 1999. Effects of growth hormone and pair-feeding on leptin mRNA expression in liver and adipose tissue. Domest. Anim. Endocrinol. 17:77–84.

Barb, C. R., G. J. Hausman, and K. L. Houseknecht. 2001. Biology of leptin in the pig. Domest. Anim. Endocrinol. 21:297–317.

Berti, L., and S. Gammeltoft. 1999. Leptin stimulates glucose uptake in C2C12 muscle cells by activation of ERK2. Mol. Cell. Endocrinol. 157:121–130.

Bolanowski, M., A. Milewicz, B. Bidzinska, D. Jedrejuk, J. Daroszewski, and E. Mikulski. 2002. Serum leptin levels in acromegaly—A significant role for adipose tissue and fasting insulin/glucose ratio. Med. Sci. Monit. 8:CR685–689.

Boni-Schnetzler, M., C. Hauri, and J. Zapf. 1999. Leptin is suppressed during infusion of recombinant human insulin-like growth factor I (rhIGF I) in normal rats. Diabetologia 42:160–166.

Budd, T. J., J. L. Atkinson, P. J. Buttery, A. M. Salter, and J. Wiseman. 1994. Effect of insulin and isoproterenol on lipid metabolism in porcine adipose tissue from different depots. Comp. Biochem. Physiol. Pharmacol. Toxicol. Endocrinol. 108:137–143.

Cammisotto, P. G., Y. Gelinas, Y. Deshaies, and L. J. Bukowiecki. 2003. Regulation of leptin secretion from white adipocytes by free fatty acids. Am. J. Physiol. Endocrinol. Metab. 285:E521–E526.

Campbell, R. G., N. C. Steele, T. J. Caperna, J. P. McMurtry, M. B. Solomon, and A. D. Mitchell. 1988. Interrelationships between energy intake and endogenous porcine growth hormone adminis-

tration on the performance, body composition and protein and energy metabolism of growing pigs weighing 25 to 55 kilograms live weight. J. Anim Sci. 66:1643–1650.

Caperna, T. J., N. C. Steele, D. R. Komarek, J. P. McMurtry, R. W. Rosebrough, M. B. Solomon, and A. D. Mitchell. 1990. Influence of dietary protein and recombinant porcine somatotropin administration in young pigs: Growth, body composition and hormone status. J. Anim. Sci. 68:4243–4252.

Ceddia, R. B., W. N. William, Jr., and R. Curi. 1998. Leptin increases glucose transport and utilization in skeletal muscle in vitro. Gen. Pharmacol. 31:799–801.

Czaja, K., M. Lakomy, W. Sienkiewicz, J. Kaleczyc, Z. Pidsudko, C. R. Barb, G. B. Rampacek, and R. R. Kraeling. 2002. Distribution of neurons containing leptin receptors in the hypothalamus of the pig. Biochem. Biophys. Res. Commun. 298:333–337.

Denis, R. G., C. Bing, E. K. Naderali, R. G. Vernon, and G. Williams. 2003. Lactation modulates diurnal expression profiles of specific leptin receptor isoforms in the rat hypothalamus. J. Endocrinol. 178:225–232.

Eden-Engstrom, B., P. Burman, C. Holdstock, and F. A. Karlsson. 2003. Effects of growth hormone (GH) on ghrelin, leptin, and adiponectin in GH-deficient patients. J. Clin. Endocrinol. Metab. 88:5193–5198.

Etherton, T. D. 2004. Somatotropic function: The somatomedin hypothesis revisited. J. Anim. Sci. 82(E-Suppl.) Online. Available: http://jas.fass.org/cgi/reprint/82/13_suppl/E239.pdf. Accessed December 1, 2004.

Fernandez-Figares, I., A. E. Shannon, D. Wray-Cahen, and T. J. Caperna. 2004. The role of insulin, glucagon, dexamethasone, and leptin in the regulation of ketogenesis and glycogen storage in primary cultures of porcine hepatocytes prepared from 60 kg pigs. Domest. Anim. Endocrinol. 27:125–140.

Fruhbek, G., J. Gomez-Ambrosi, and J. A. Martinez. 1999. Pre- and postprandial expression of the leptin receptor splice variants OB-Ra and OB-Rb in murine peripheral tissues. Physiol. Res. 48:189–195.

Guo, K. Y., P. Halo, R. L. Leibel, and Y. Zhang. 2004. Effects of obesity on the relationship of leptin mRNA expression and adipocyte size in anatomically distinct fat depots in mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 287:R112–R119.

Houseknecht, K. L., C. P. Portocarrero, S. Ji, R. Lemenager, and M. E. Spurlock. 2000. Growth hormone regulates leptin gene expression in bovine adipose tissue: Correlation with adipose IGF-1 expression. J. Endocrinol. 164:51–57.

Iglesias, P., J. J. Diez, M. J. Fernandez-Reyes, M. A. Bajo, A. Aguilera, J. Mendez, R. Codoceo, and R. Selgas. 2002. Effects of short-term recombinant human growth hormone therapy on plasma leptin concentrations in dialysis patients. Nephrol. Dial. Transplant 17:260–264.

Isozaki, O., T. Tsushima, M. Miyakawa, Y. Nozoe, H. Demura, and H. Seki. 1999. Growth hormone directly inhibits leptin gene expression in visceral fat tissue in fatty Zucker rats. J. Endocrinol. 161:511–516.

Leininger, M. T., C. P. Portocarrero, A. P. Schinckel, M. E. Spurlock, C. A. Bidwell, J. N. Nielsen, and K. L. Houseknecht. 2000. Physiological response to acute endotoxemia in swine: Effect of genotype on energy metabolites and leptin. Domest. Anim. Endocrinol. 18:71–82.

Lin, J., C. R. Barb, R. R. Kraeling, and G. B. Rampacek. 2003. Growth hormone releasing factor decreases long form leptin receptor expression in porcine anterior pituitary cells. Domest. Anim. Endocrinol. 24:95–101.

Lin, J., C. R. Barb, R. L. Matteri, R. R. Kraeling, X. Chen, R. J. Meinersmann, and G. B. Rampacek. 2000. Long form leptin receptor mRNA expression in the brain, pituitary, and other tissues in the pig. Domest. Anim. Endocrinol. 19:53–61.

Louveau, I., H. Quesnel, and A. Prunier. 2000. GH and IGF-I binding sites in adipose tissue, liver, skeletal muscle and ovaries of feed-restricted gilts. Reprod. Nutr. Dev. 40:571–578.

Malmlof, K., and T. Johansen. 2003. Growth hormone-mediated breakdown of body fat: Insulin and leptin responses to GH are modulated by diet composition and caloric intake in old rats. Horm. Metab. Res. 35:236–242.

Montague, C. T., J. B. Prins, L. Sanders, J. E. Digby, and S. O'Rahilly. 1997. Depot- and sex-specific differences in human leptin mRNA expression: Implications for the control of regional fat distribution. Diabetes 46:342–347.

Ono, Y., M. B. Solomon, C. M. Evock-Clover, N. C. Steele, and K. Maruyama. 1995. Effects of porcine somatotropin administration on porcine muscles located within different regions of the body. J. Anim. Sci. 73:2282–2288.

Otte, C., J. M. Otte, D. Strodthoff, S. R. Bornstein, U. R. Folsch, H. Monig, and S. Kloehn. 2004. Expression of leptin and leptin receptor during the development of liver fibrosis and cirrhosis. Exp. Clin. Endocrinol. Diabetes 112:10–17.

Potter, J. J., L. Womack, E. Mezey, and F. A. Anania. 1998. Transdifferentiation of rat hepatic stellate cells results in leptin expression. Biochem. Biophys. Res. Commun. 244:178–182.

Raman, P., S. S. Donkin, and M. E. Spurlock. 2004. Regulation of hepatic glucose metabolism by leptin in pig and rat primary hepatocyte cultures. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286:R206–R216.

Ramsay, T. G. 2000. Porcine leptin alters insulin inhibition of lipolysis in porcine adipocytes in vitro. J. Anim. Sci. 79:653–657.

Ramsay, T. G. 2003a. Porcine leptin inhibits lipogenesis in porcine adipocytes. J. Anim. Sci. 81:3008–3017.

Ramsay, T. G. 2003b. Porcine leptin inhibits protein breakdown and stimulates fatty acid oxidation in C2C12 myotubes. J. Anim. Sci. 81:3046–3051.

Ramsay, T. G., C. Morrison, and X. Yan. 1998. The obesity gene in swine: Sequence and expression of porcine leptin. J. Anim. Sci. 76:484–490.

Ramsay, T. G., and M. P. Richards. 2004. Hormonal regulation of leptin and leptin receptor expression in porcine subcutaneous adipose tissue. J. Anim. Sci. 82:3486–3492.

Raver, N., E. E. Gussakovsky, D. H. Keisler, R. Krishna, J. Mistry, and A. Gertler. 2000. Preparation of recombinant bovine, porcine, and porcine W4R/R5K leptins and comparison of their activity and immunoreactivity with ovine, chicken, and human leptins. Protein Expr. Purif. 19:30–40.

Rentsch, J., and M. Chiesi. 1996. Regulation of ob gene mRNA levels in cultured adipocytes. FEBS Lett. 379:55–59.

Richards, M. P., and S. M. Poch. 2002. Quantitative analysis of gene expression by reverse transcription polymerase chain reaction and capillary electrophoresis with laser-induced fluorescence detection. Mol. Biotechnol. 21:19–37.

Rule, D. C., S. B. Smith, and H. J. Mersmann. 1989. Glycerolipid biosynthesis in porcine adipose tissue in vitro: Effect of adiposity and depot site. J. Anim. Sci. 67:364–373.

Salfen, B. E., J. A. Carroll, and D. H. Keisler. 2003. Endocrine responses to short-term feed deprivation in weanling pigs. J. Endocrinol. 178:541–551.

Schulz, C., I. Wieczorek, K. Reschke, and H. Lehnert. 2002. Effects of intracerebroventricularly and intraperitoneally administered growth hormone on body weight and food intake in fa/fa Zucker rats. Neuropsychobiology 45:36–40.

Shintani, M., H. Nishimura, S. Yonemitsu, H. Masuzaki, Y. Ogawa, K. Hosoda, G. Inoue, Y. Yoshimasa, and K. Nakao. 2000. Down-regulation of leptin by free fatty acids in rat adipocytes: Effects of triacsin C, palmitate, and 2-bromopalmitate. Metabolism 49:326–330.

Spurlock, M. E., M. A. Ranalletta, S. G. Cornelius, G. R. Frank, G. M. Willis, S. Ji, A. L. Grant, and C. A. Bidwell. 1998. Leptin expression in porcine adipose tissue is not increased by endotoxin but is reduced by growth hormone. J. Interferon Cytokine Res. 18:1051–1058.

Steele, N. C., J. P. McMurtry, R. G. Campbell, T. J. Caperna, and R. W. Rosebrough. 1995. Effect of dietary energy intake and exogenous porcine growth hormone administration on circulating porcine growth hormone concentration and response to human growth hormone-releasing factor administration in growing swine. Domest. Anim. Endocrinol. 12:293–298.

Tena-Sempere, M., L. Pinilla, F. P. Zhang, L. C. Gonzalez, I. Huhtaniemi, F. F. Casanueva, C. Dieguez, and E. Aguilar. 2001. Developmental and hormonal regulation of leptin receptor (Ob-R) messenger ribonucleic acid expression in rat testis. Biol. Reprod. 64:634–643.

Wang, J., L. Rong, M. Hawkins, N. Barzilai, and L. Rossetti. 1998. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 393:684–688.

Whisnant, C. S., and R. J. Harrell. 2002. Effect of short-term feed restriction and refeeding on serum concentrations of leptin, luteinizing hormone and insulin in ovariectomized gilts. Domest. Anim. Endocrinol. 22:73–80.

Zhang, Y., K. Y. Guo, P. A. Diaz, M. Heo, and R. L. Leibel. 2002. Determinants of leptin gene expression in fat depots of lean mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282:R226–R234.